PB86-164894

Enzyme-Linked Immunoassays for the Detection of Microbial Antigens and Their Antibodies

Massachusetts Univ. Medical School, Worcester

Prepared for

Health Effects Research Lab.
Research Triangle Park, NC

Mar 86

U.S. Department of Commerce
National Technical Information Service
NTIS

PB86-164894

EPA/600/D-86/051
March 1986

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by

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EPA Grant CR810303

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SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA *(Please read Instructions on the reverse before completing)*		
1. REPORT NO. EPA/600/D-86/051	2.	3. RECIPIENT'S ACCESSION NO. PB86 164894AS
4. TITLE AND SUBTITLE ENZYME-LINKED IMMUNOASSAYS FOR THE DETECTION OF MICROBIAL ANTIGENS AND THEIR ANTIBODIES		5. REPORT DATE March 1986 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) John E. Herrmann		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Univ. of Massachusetts Medical School Division of Infectious Diseases Worcester, Massachusetts 01605		10. PROGRAM ELEMENT NO. CBMC1A 11. CONTRACT/GRANT NO. CR810803
12. SPONSORING AGENCY NAME AND ADDRESS Health Effects Research Laboratory (HERL) - RTP, NC Office of Research and Development (ORD) U.S. Environmental Protection Agency (USEPA) Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Book Chapter 14. SPONSORING AGENCY CODE EPA/600/11
15. SUPPLEMENTARY NOTES P.O. Elmer W. Akin, TMD/HERL/ORD/USEPA (CI)		

16. ABSTRACT

The advantages of enzyme-immunoassay (EAI) over radioactive assay techniques are mainly convenience in use, in that the labelled immunoreagents are stable for long periods, and the precautions and disposal procedures required for radioisotopes are unnecessary. In addition, the use of chromogenic substrates for the enzyme labels permits visual interpretation of test results in some cases. The only real disadvantages of EIA tests are the loss of antibody reactivity that may result from conjugation to enzymes, and the limits of substrate detection. For example, use of enzymes that have molecular weights higher than that of IgG molecules such as B-D-galactosidase (mw 540,000 daltons) can cause steric hindrance of antibody activity. With regard to limits of substrate detection, improvement of enzyme detection by use of fluorogenic, luminescent, or radioactive substrates has been proposed. The major emphasis of this report will be on current developments in EIA methodology and the application of EIA to diagnosis of infectious diseases. This includes tests for both antigen and antibody detection in viral, rickettsial, bacterial, and mycotic infections. EIA tests for diagnosis of parasitic agents, hormones, and other antigens have been described but are not discussed here.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSFIED	52
	20. SECURITY CLASS *(This page)* UNCLASSFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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Enzyme-Linked Immunoassays for the Detection of Microbial Antigens and their Antibodies

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I. Introduction

Enzyme-immunoassay (EIA) evolved as a result of the findings by Nakane and Pierce (1966) that antibodies could be labelled with enzymes for use in histochemical staining procedures, and by Catt and Tregear (1967) who described solid-phase radioimmunoassays (RIA). The substitution of enzyme labels for radioactive ones in the solid-phase RIA resulted in solid-phase EIA tests for human chorionic gonadotropin (Van Weeman and Schuurs, 1971) and for IgG detection (Engvall and Perlmann, 1971). The latter authors coined the term "enzyme-linked immunosorbent assay (ELISA)" for solid-phase EIA tests.

Initial solid-phase EIA tests were not as sensitive as the corresponding RIA, but improvement in enzyme labelling techniques have made the two types of assays comparable for detecting a number of antigens and antibodies. In some systems where RIA and EIA have been directly compared, there is little difference in the sensitivity or specificity of the two assays (Sarkkinen, et al. 1981c).

The advantages of enzyme labels over radioactive ones are mainly convenience in use, in that the labelled immunoreagents are stable for long periods, and the precautions and disposal procedures required for radioisotopes are unnecessary. In addition, the use of chromogenic substrates for the enzyme labels permits visual interpretation of test results in some cases. The only real disadvantages of EIA tests are the loss of antibody reactivity that may result from conjugation to enzymes, and the limits of substrate detection. For example, use of enzymes that have molecular weights

higher than that of IgG molecules such as B-D-galactosidase (mw 540,000 daltons) can cause steric hindrance of antibody activity (Herrmann and Morse, 1974). With regard to limits of substrate detection, improvement of enzyme detection by use of fluorogenic, luminescent, or radioactive substrates (reviewed by Yolken, 1982) has been proposed.

The general principles of EIA tests and details of earlier studies have been reviewed a number of times (Yolken, 1980;Yolken 1982; Hildebrand 1979, Voller, et al. 1981) and will not be repeated in detail here. Rather, the major emphasis will be on current developments in EIA methodology and the application of EIA to diagnosis of infectious diseases. This will include tests for both antigen and antibody detection in viral, rickettsial, bacterial, and mycotic infections. EIA tests for diagnosis of parasitic agents, hormones, and other antigens have been described but will not be discussed here.

II. Design of Enzyme Immunoassays

A. Solid-phase assays

The solid-phase or heterogeneous EIA requires immobilization of antigens or antibodies to a solid surface as a means of separating antigen-antibody complexes. Solid-phase surfaces used to immobilize antigens or antibodies have for most applications been polystyrene beads, tubes, and wells of microtiter plates, or wells of polyvinyl chloride microtiter plates. Coupling of proteins to these surfaces is usually done by passive adsorption. More recently, adsorption of antigens or antibodies to nitrocellulose membranes has been adapted to detection of viruses by EIA (Bode, et al. 1984). Covalent linkage of antibodies or antigens to a variety of surfaces, including porous glass (Lynn,1975) nylon (Hendry and Herrmann, 1980), cellulose (Ferrua, et al., 1979), agarose (Streefkerk and Deelder, 1975), and polyacrylamide (Avrameas and Guilbert, 1971) has been described.

The majority of solid-phase EIA tests that have been found to be clinically useful utilize plastic microtiter-plates or beads, with antigen or antibody passively adsorbed to the solid phase. In situations where a given antigen does not readily attach, antibody to the antigen is applied first. For some antigens, non-specific adsorbents such as poly-L-lysine have been used to enhance antigen adsorption, or the Clq component of complement to capture antigen-antibody complexes (Yolken, 1982). Most of the assays described are of the non-competitive type, although a number of competitive assays have been described. The disadvantage of many competitive assays for antigen detection is that labelled antigen is used which is usually more difficult to prepare than labelled antibody. With the advent of monoclonal antibodies, competitive assays for specific antibodies are becoming more common. A number of different formats of EIA tests for antigens and antibodies are possible. These are discussed in sections II C and II D.

B. Homogeneous assays

To avoid the need for separation of antigen-antibody complexes, homogeneous EIA tests were developed (Rubinstein, et al. 1972). Homogeneous assays are based on reaction of antigen with an antibody-enzyme complex. This results in steric hindrance of the enzyme, which cases a decrease in product after reaction with enzyme substrate. The major advantage of homogeneous assays is that they do not require the separation and washing steps required in heterogeneous assays. The major disadvantage of this type of EIA is that it has been difficult to apply it to detection of high molecular weight antigens with the degree of sensitivity required. Thus, the homogeneous assay has been used mainly for detection of hormones, drugs, and other low-molecular weight substances. Means to improve the sensitivity of homogeneous assays have been devised (review by Yolken, 1932) but have not been widely used. Thus, the applications discussed below will be limited to

EIA tests of the solid-phase type.

C. Types of assays for antibodies

Enzyme-immunoassays for antibodies to microbial agents have been utilized for most all of the common infectious diseases, because the sensitivity required is well within the range of EIA. A summary of the procedures most often used are given in Table I. The choice of assay depends on the sensitivity required, the availability of reagents, and whether a class-specific test is desired. For detection of IgM, either the non-competitive EIA or class capture methods can be used. In the non-competitive EIA, enzyme-labeled antiglobulin (step 3) specific for IgM is used. The advantage of IgM capture methods is that there is less problem with sera containing rheumatoid factor (RF), although precautions must be taken in designing the test to avoid weakly reactive sera containing RF (Parry, 1984; Briantais, et al. 1984).

The use of enzyme labeled antigen in tests for IgM has been useful for diagnosis of a number viral infections, e.g., cytomegalovirus (Schmitz, et al. 1980), Epstein-Barr virus (Schmitz, 1982) and flaviviruses (Schmitz and Emmerich, 1984) but requires purified antigen for labelling. Thus, it is limited to those agents where production and purification of antigen is relatively simple and offers improved diagnosis as well.

The majority of class-specific EIA tests are for IgM (sometimes referred to as MAC-ELISA for M antibody capture-ELISA), because IgM is the most important serological marker indicating recent infection when only single serum specimens are available. However, substitution of other class-specific markers can be used.

D. Assays for Antigen Detection

Methods for detecting antigen by EIA can be done by competitive and non-competitive formats, as with assays for antibodies. The lower limit of

sensitivity for detecting antigen by EIA in most studies is approximately 100 pg to 1 ng, although lower limits have been described (Kato, et al. 1975). This level of sensitivity is sufficient to detect virtually all culture-propagated infectious agents, but is not always sensitive enough to detect antigen directly in clinical specimens.

The types of formats most often used are shown in Table II.

III. Factors in Sensitivity and Specificity

A number of factors determine how efficient an assay is in detecting antigens and antibodies. Some are inherent and cannot be controlled, eg. the amount of antigen that is usually present in a positive clinical specimen, and others, such as test design, can be controlled. Some of the more important variables that can be controlled are discussed here.

a. Enzyme, Substrates, and Labelling Procedures

Most EIA formats require covalent coupling of enzymes to antibody or antigen. A number of enzymes and coupling techniques have been tried (reviewed by Yolken, 1982). The most consistent results have been obtained with horseradish peroxidase coupled by use of periodate (Nakane and Kawaoi, 1974) and alkaline phosphatase coupled by use of glutaraldehyde (Avrameas, 1969). Most of the assays found to be clinically useful in diagnosing infectious diseases use chromogenic substrates, although fluorogenic and radioactive substrates have been described (Yolken, 1982; Avrameas and Guesdon, 1982), as have luminescent ones (review by Seitz, 1984).

A more recent development in EIA which has found application in diagnostic microbiology is the use of avidin and biotin (Guesdon, et al, 1979). The test is based on the high affinity complex formed between biotin and avidin. The most common method is to use specfiic antibody labelled with biotin; the indicator system is enzyme-labelled avidin. A recent adaptation of this method using biotinillated beta-lactamase in combination with avidin was effective for detecting rotavirus antigen (Yolken and Wee, 1984).

B. Antibody Immobilization

In addition to antibody affinity and the sensitivity of the indicator system used, the sensitivity of many antigen detection systems de-

pends on the amount of antibody that can be effectively immobilized on a solid phase. Methods for immobilization of antibody on plastic surfaces are usually based on simple adsorption, although convalent-linking methods have been utilized. The amount of immunoglobulin that can be immobilized on various plastics is given in Table III (Herrmann, 1981). Increasing the amount of antibody bound to a solid phase surface should result in increased sensitivity of the EIA. However, it has been noted that increasing the concentration of antibody for coating surfaces beyond 10 ug/ml does not give an increase in immunoassay sensitivity. This is apparently due to desorption of antibody from the plastic surface, and steric hindrance of antibody that is adsorbed.

Solid-phase surfaces other than plastic tubes, beads, or particles, such as porous glass (Lynn, 1975) have been used to immoblize antibody. However, the majority of EIA tests for microbial infections use either plastic plates or beads. A more recent development mentioned above that may be applicable to a variety of microbial antigens and antibodies is the use of nitrocellulose membrane discs as a solid phase. This was successfully developed as a visual-readout method for detecting adenovirus antigens and antibodies (Bode, et al., 1984).

C. Immunoreagents

The use of antibodies that are highly specific and have high affinity is the most critical aspect of most EIA techniques. An example of how the reagents used determines EIA effectiveness was shown in the two papers discussed below on detection of Clostridium deficile toxin, where the sensitivity was increased from 58.6% to 95% by changing the immunoreagents used (Laughon et al., 1984). The diluents used for antigen preparation can also alter the sensitivity. Disrupting microbial agents with detergents or other chemicals may increase the sensitivity of some assays but decrease others

(Yolken, 1982). A number of diluents not usually used in EIA tests was tested by Conroy and Esen (1984) for adsorbing a plant protein to polystyrene. These included detergents, acids, alcohols and urea. Use of alcohols or urea increased the EIA substantially. Whether this would be applicable to microbial antigens remains to be tested.

IV. Applications of Assays for Microbial Antibodies

The diagnosis of infectious disease has been accomplished in the past by either isolation of the infectious agent, or by measuring serological conversion to a given agent. Serological conversion can be measured by a high level of IgM, or by an increase in total antibody in convalescent sera compared with acute sera. The use of EIA is an extension of prevously used serological tests, using enzyme-labelled antibody or antigen to determine antibody content. Direct detection of antigen by EIA represents a more dramatic departure from previous methods based on culture. Also, the method has enabled detection of infectious for agents which are difficult to cultivate, such as hepatitis A virus and rotavirus, or agents which cannot be cultivated, such as hepatitis B virus (Section V).

A. Bacterial and mycotic infections

A summary of the bacterial and mycotic infections for which EIA serological tests have been devised is given in Table IVA. Because EIA techniques are not difficult to develop for antibody detection, there are an even increasing number of tests reported. However, developing tests for antibody which have consistent diagnostic accuracy can be difficult and the tests cited in Table IVA report various degrees of sensitivity and specificity. In a review by Hill a than 95%, but the sensitivity is as low as 50% in many cases. Thus, each test must be carefully examined to determine how useful it is for a specific infection. In addition to

problems with low sensitivity, the major drawback to many assays is the lack of standardization. Without the availability of standard serum samples for evaluation of assays, new assays require testing by a number of investigators before their validity can be assessed. In many instances, however, serological diagnosis is the only means available to many laboratories for diagnosing some of the more exotic diseases. For example, for diagnosis of Lyme disease, culture of the causative spirochete is possible, but is often ineffective. Use of an EIA test for specific IgM and IgG response in patients with proven Lyme disease was diagnostic in 11 of 12, and the EIA gave no false positive results in 40 control subjects (Craft, et al, 1984). Early recognition of disease is another area where EIA tests for specific IgM and IgG may be the best or only method available to some, such as diagnosis of tuberculous meningitis (Hernandez, et al., 1984), although newer developments in use of latex particle agglutination for diagnosis of this disease may prove more useful than serology (Krambovitis, et al., 1984).

B. Viral and Rickettsial Infections

The applications of antibody EIA for diagnosis of viral and rickettsial infections are given in Table IVB. As discussed above for bacterial and mycotic infections, serological diagosis by EIA for most of the agents listed is still largely experimental. The most frequently used applications are in screening for immune status, such as rubella testing, and for cytomegalovirus antibody,and for antibodies to Hepatitis B antigens. Serological diagnosis of infectious mononucleosis is also the method of choice. The heterophile antibody test used is not specific for Epstein-Barr virus, and the test is usually done by agglutination, but EIA tests described may be more sensitive (Halbert, 1982). Specific EIA tests for antibody to Epstein-Barr virus components have also been described (Hopkins, et al. 1982) but are not yet widely used.

Because many viruses are difficult to isolate, or haven't yet been cultivated, serological tests are often the most useful for diagnosis. The use of IgM capture EIA for determining recent viral infection is becoming more common, and may provide aid in diagnosis where antigen detection methods are not available.

V. Application of Assays for Microbial Antigens

The use of EIA tests for detection of microbial antigens provides an alternative to culture as a means for direct identification of a specific microbial agent. It also provides a means to detect microbial agents which have not been successfully propagated. The detection of circulating antigen or detection of anitgen in other body fluids by EIA is more difficult than detection of antibody because of the sensitivity required, and because of interfering substances in specimens such as feces and respiratory secretions. For this reason, very few antigen detection assays have the sensitivity and specificity required to be used as a primary diagnostic test. The number of tests that have been developed, however, is impressive and because of the possibilities for rapid, specific diagnosis, the interest in antigen detection by EIA remains high.

A. Bacterial and Mycotic Infections

The tests developed for bacterial infections are primarily for diseases which have causative agents difficult to culture, or where rapid diagnosis will permit prompt treatment. As can be noted by comparing Tables IVA and V A, there are far fewer EIA antigen detection tests than antibody tests, for reasons cited above. The efficiencies of the assays reported are variable, but none are as sensitive as the corresponding culture technique. Only one test is commercially available at this writing, an EIA for detecting N. gonorrhoeae antigens. The EIA has been evaluated by a number of laboratories (Table V A). In general, almost all reports have found that the EIA was

equivalent to culture for detection of gonorrhoeae in males. In females, sensitivities have ranged from 74.4% (Papasian, et al, 1984) to 90.9% (Danielsson, et al, 1983) and specificities from 86.5% (Manis, et al., 1984) to 100% (Danielson, et al., 1983). Two other extensive studies reported specificities of 98% (Stamm, et al, 1984; Demetriou, et al, 1984). Thus, the reliability of EIA appears to depend on the efficiency of the culture method used, and perhaps variability in performing the test itself.

EIA tests have also been developed for another agent of sexually-transmitted disease, Chlamydia trachomatis. This agent is more difficult to cultivate than N. gonorrhoeae, in that cell cultures are required. Only one EIA has been examined with a significant number of samples, a commercially-produced test under development (Chlamydiazyme, Abbott Laboratories). Pre-market evaluation of this test on 416 patients showed the EIA had a sensitivity of 83% (63/76) and a specificity of 94% (Jones et al. 1984). In a larger study involving 2384 specimens the EIA had a sensitivity of 83% and a specificity of 94% (Herrmann, et al., 1983).

Mycoplasma hominis may also be involved in sexually-transmitted disease, and diagnosis by culture requires expertise. An antigen EIA has been developed (Miettinen, et al., 1984) and was positive for 6 specimens positive by culture. More extensive evaluation is required to determine the utility of the assay as a screening procedure.

Detection by EIA of bacterial antigens in cerebrospinal fluid (CSF) and respiratory tract secretions has also been attempted, with good results for some antigens. Yolken, et al. (1984) reported 100% sensitivity for detecting pneumococcal antigen in 25 CSF specimens, but others have found difficulty in differentiating pneumococcal antigens from meningococcal antigens in CSF by EIA (Sippel, et al., 1984). Use of monoclonal antibody for detecting group A meningococcal antigens has been described (Sugasawara, et al.,

1984) but was 84% as sensitive as polyclonal serum for detecting antigen in the same CSF samples. Detection of Haemophilous influenzae B by EIA has been shown to be effective in limited clinical trials. Drow, et al. (1979) developed an EIA which was 100% sensitive on 11 positive CSF samples, and Sippel, et al. (1984), using a similar EIA system detected 17 of 20 samples that were positive for Haemophilus by counterimmunoelectrophoresis or coagulation. There were 17 positive by culture. Antigen detection by EIA for respiratory infections has been described for Legionella pneumophila, tuberculosis, and streptococcus group A infection. The most extensive study for detecting L. pneumophila antigens in urine was done by Sathapatayavongs, et al. (1982) who obtained a 82.9% (39/47) sensitivity and a specificty of 100% in 178 urines from patients with other diseases. An inhibition EIA for detection of streptococcus group A antigen is throat swabs was also effective, giving a sensitivity of 97.0% and a specificity of 97.9% (Knigge, et al., 1984). Confirmed diagnosis of tuberculosis is difficult because of the long period required to culture the causative organism. Preliminary results of an EIA developed by Sada, et al. (1983), utilizing rabbit antibody to BCG, showed a sensitivity of 81.2% in 16 samples from patients with tuberculosis meningitis. Because antibodies to BGG may cross react with other mycobacteria, as well as Nocardia and Corynebacterium sp., the test needs further evaluation for specificity.

There have also been EIA tests developed for detection of bacterial toxins in clinical samples, most notably assays for Clostridium difficile toxins. Lyerly, et al., (1983) developed an EIA for Cl. difficile A toxin which was 100% specific in 31 samples, but only 58.6% (17/29) sensitive. An improved assay for this toxin and for B toxin was reported by Laughon, et al. (1984). Of 79 tissue-culture positive specimens, 91% were positive for toxin A and 80% were positive for toxin B. Combined, 95% were positive for either

A or B toxin. Thus, this is one EIA test that appears to be a marked improvement over the difficult tissue culture toxin assay. Detection by EIA in stool of toxins from other Clostridium species, Cl.perfringens A (McClane and Strouse, 1984) and Cl. botulinum A and B (Dezfulian, et al., 1984) has also been reported.

Two other unrelated infections have been diagnosed by antigen EIA, Bacteroides fragilis and Yersinia pestis infections. A test for B. fragilis in urine was 100% specific, and detected antigen in 73% (11/15) of individuals shown to be infected with B. fragilis (Rissing, et al., 1984). Use of monoclonal antibody against the F1 antigen of Y.pestis was insensitive in an EIA, detecting antigen in 20% (2/10) sera from patients with acute bubonic plaque (Williams, et al., 1984).

B. Viral Infections

The interest in EIA methods for rapid diagnosis of viral infections has been high, because of the time and expense required for isolation of the agents in cell culture. Further, some viruses cannot be cultivated or are difficult to cultivate. The latter includes Hepatitis A and B viruses, and rotavirus. Tests for Hepatitis B surface antigen and e antigen have been commercially available for some time, have been extensively evaluated, and need not be elaborated on here. Detection of Hepatitis A antigen by EIA has also been reported (Mathiesen, et al., 1978, Locarini, et. al., 1978) but is not at this time commercially availalbe. The sensitivity of the EIA developed by Mathiesen, et al. was 77% (10/13) compared with immune electron microscopy (IEM). Locarini, et al. were able to detect Hepatitis A in 85% (17/20) of samples positive by IEM, and found no false positive EIA reactions in fecal samples from patients with Hepatitis B or Non A-Non-B hepatitis virus infections.

In addition to detection by EIA of Hepatitis viruses, the EIA tests

most frequently developed have been for respiratory viruses, herpes viruses, and gastroenteritis viruses (Table VB).

1. Respiratory Viruses

Because antiviral agents are becoming available for some of the respiratory virus infections, rapid methods of diagnosis are essential for prompt treatment. Rapid diagnosis by EIA has been proposed for a number of respiratory viruses. Several have been described for diagnosis of respiratory syncytial virus. Specificity does not appear to be a problem with any EIA reported, but the sensitivity is less than that found by culture. Compared with culture, sensitivities have been found to be 79.3% (23/29) (Chao, et al. 1979), 60.9% (25/41) (Hornsleth, et al., 1981), 78.7% (37/47), (Hornsleth, et al., 1982), and 82.8% (77/93) (McIntosh, et al., 1982).

Diagnosis of viral influenza by EIA has also been reported, with variable results. Compared with culture, Harmon and Pawlik (1982) reported a sensitivity of 53% (21/40). A later report by Harmon, et al. (1983) on an EIA using fluorogenic substrates gave a sensitivity of 87% (27/31). By use of a radioactive substrate, Coonrod, et al., (1984) were able to detect influenza virus in nasal washes, but the maximum sensitivity at any given day of infection was 48% (12/25). A similar assay described by Yolken (1980) on samples from 12 volunteers gave sensitivities of 78 to 100%, depending on the day tested. Tests for adenovirus in respiratory secretions have also been developed. Harmon and Pawlik (1982) compared an EIA with tissue culture isolation and were able to detect by EIA 62% (13/21) of adenovirus-positive specimens.

2. Herpesviruses

The interest in sexually-transmitted herpes virus and the availability of treatment have led to the development of a number of EIA

tests for rapid diagnosis of herpes infection. Most lack sufficient sensitivity to be used as a substitute for culture. Two evaluations of a commercial EIA for herpes genital infection (Ortho Diagnostic Systems, Inc.) have been reported. Morgan and Smith (1984) found the test to be 71.9% (105/146) sensitive and 100% specific in 366 control specimens. Warford et al. (1984) however, found the test to be only 52.5% (155/295) sensitive and 96.9% (834/860) specific. Some of the other EIA tests developed have given similar results. Lawrence, et al., (1984) developed an EIA which was 50.5% (94/186) sensitive and 99.1% (423/427) specific. An EIA reported by Grillner and Landquist (1983) was 75.9% (44/58) sensitive and 100% specific. Two assays reported appear to have higher efficiencies. Nerurkar et al. (1984), using a biotin-avidin EIA obtained a 95.6% sensitivity and a 91.4% specificity; Land, et al. (1984), using a detergent-treated specimen obtained a sensitivity and specificity of 94%.

Development of EIA tests for other viruses in the hepresivurs group have also been reported. Ziegler (1984) developed an EIA for varicella-zoster viral antigens which detected 8/8 culture positive specimens, and Pronovost, et al. (1982) developed a chemiluminescent EIA for cytomegalovirus antigen which detected 9/11 culture-positive specimens

3. Gastroenteritis viruses

The two most important gastroenteritis viral agents for which EIA tests have been developed are rotavirus and enteric adenoviruses. An EIA test for rotavirus was first develoed by Yolken, et al. (1977), and commercial assays are now available. A recent evaluation of two commercial products (Rotazyme, Abbott Laboratories, and Enzymgnost, Behring) showed the sensitivity of Rotazyme to be 88% and Enzygnost, 98% (Morinet, et al., 1984). The standard for comparison was electron microscopy (EM). Both EIA methods appear suitable for use if EM is not available, although Rotazyme is known to

cause false-positive results in samples from neonates (Krause, et. al. 1983; Chrystie, et al., 1983) and is insensitive in samples from adults (Herrmann, et al., 1985, in press).

Enteric adenoviruses (types 40 and 41) are difficult to isolate, therefore EIA methods would be preferable if the sensitivity was satisfactory. Preliminary results from Johansson, et al. (1980) suggest that development of an EIA specific for enteric adenoviruses is possible.

VI Use of Monoclonal Antibodies

The use of monoclonal antibodies in EIA tests offers two potential advantages 1) improved specificity due to the nature of monoclonal antibodies and 2) improved sensitivity by allowing for clearer EIA cut-off values. Sensitivity could also be increased by increasing the amount of detector antibody used in an EIA. However, because monoclonal antibodies react with only one epitope of a given antigen, more than one monoclonal antibody may be needed to achieve the desired sensitivity. In practice, monoclonal antibodies have been used successfully in latex agglutination tests and also in immunofluorescence technqiues (Nowinkski, et al., 1983). Their use in EIA has been limited to date, but the number of ones described for microbial antigens to date suggest that applications in clinical diagnosis will be increasing. The EIA tests that have been developed look promising. For EIA detection of adenovirus group antigens in stools, monoclonal antibodies were as sensitive as polyclonal ones, and were more sensitive for detecting non-cultivatable adenoviruses (presumably enteric serotypes). All 12 stool samples positive by EM were monoclonal EIA positive (Anderson, et al., 1983).

Use of monoclonal antibodies for detection by EIA of microbial antigens in cerebrospinal fluid (CSF) also look promising. In a comparison of polyclonal and monoclonal EIA tests for group A meningococcal antigens in CSF, 21

of 25 CSF specimens positive by polyclonal EIA were positive by monoclonal EIA (Sugasawara, et al., 1984). In a preliminary study, 5/5 CSF specimens positive for group B streptococcal antigen reacted in a monoclonal EIA (Morrow, et al. 1984).

For diagnosis of rotavirus infection, we have found that a monoclonal EIA was 100% sensitive and specific for samples from adults and neonates as well as young children (Herrmann, et al. 1985, in press). This was possible due to the high affinity and broad group specificity of the monoclonal antibody used (Cukor, et al., 1984).

Preliminary results of a monoclonal EIA for diagnoses of Legionellosis showed positive correlations in 3/3 cases (Bibb, et al., 1984). However, in a preliminary study on using a monoclonal EIA for diagnosis of bubonic plaque, only 2 of 10 were positive (Laughon, et al., 1984). In this situation, where the sensitivity is low, use of polyclonal sera in a control EIA would be desirable. This would help determine if the problem was the monoclonal antibody or the amount of antigen present in the clinical sample.

VII. Future Prospects

Although many of the current EIA tests for microbial antigens and antibodies have not realized their potential, there are reasons to believe this situation will improve. For detection of antibodies, the major problems are standardization of reagents and EIA methodology. This should improve when more reagents become commercially available, and when a standard method is selected from the variety of procedures now available. For detection of antigen, which offers a rapid and direct means of diagnosing microbial infections, the major problem has been lack of sensitivity. Increasing the sensitivity of polyclonal EIA tests by using more concentrated immunoreagents or more sensitive enzyme substrates has often resulted in a loss of specificity.

From the reports available to date, it appears that use of the appropriate monoclonal antibodies may solve the problem of sensitivity for detecting many infectious agents in clinical samples. With the increasing number of monoclonal antibodies available for varius microbial antigens, we can expect that more of them will be utilized for EIA detection systems. If the affinities of monoclonal antibodies can be increased, the EIA tests may be sufficienty sensitive.

Another approach which is being taken for rapid diagnosis is the use of nucleic-acid probes in hybridization techniques (review by Richman, et al., 1984). To date, most of the probes have used radioactive labels (^{32}P) and require one or two days for assay which makes them impractical for clinical laboratories. The use of biotin labels coupled with enzyme markers may improve both the speed of the assay and the sensitivity of the assay (Richman, 1984). Whether this technique will become useful and practical for direct detection of microorganisms in clinical specimens remains to be determined.

Acknowledgements

This work was supported by contract DAMD 17-83-C3087 from the U.S. Army Medical Research and Development Command, by Cooperative Agreement CR 8-10803-01 from the U.S. Environmental Protection Agency, and by a grant from the World Health Organization.

The research described in this paper has been peer and administratively reviewed by the U.S. Environmental Protection Agency and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Table I

Methods for Antibody EIA

EIA Type/Procedure	
Non Competitive EIA	**Competitive EIA**
1. Specific antigen is attached by passive adsorption or with specific antibody	1. Same as non-competitive EIA
2. Test serum or solution containing specific antibody is added.	2. Test serum, or solution plus enzyme-labeled specific antibody is added.
3. Enzyme-labeled antiglobulin is added	3. Enzyme substrate is added
4. Enzyme substrate is added	4. Substrate hydrolysis product is inversely proportional to the amount of antibody present
5. Substrate hydrolysis product is proportional to the amount of antibody present	
Class specific capture EIA	
Labelled Antigen EIA	**Labelled Antibody EIA**
1. Solid phase is coated with IgM specific anti-IgM antibody	1. Same as labelled antigen EIA

Table 1, Continued

2. Test serum or solution containing IgM is added	2. Same as labelled antigen EIA
3. Enzyme-labelled specific antigen is added	3. Specific antigen is added
4. Enzyme substrate is added	4. Antisera specific for antigen is added (unlabelled for indirect test, enzyme-labelled for direct test).
5. Substrate hydrolysis product is proportional to the amount of IgM present	5. If indirect test, enzyme-labelled antiglobulin is added
	6,7. Same as 4,5 for labelled antigen EIA

Table II

Assays for Antigen Detection

EIA type/procedure	
Non-competitive EIA	
Direct EIA	**Indirect EIA**
1. Antigen-specific antibody is attached to solid phase	1. Same as direct EIA
2. Test specimen containing antigen is added	2. Same as direct EIA
3. Enzyme-labelled specific antibody is added	3. Specific antibody (prepared in species different from that used in step 1) is added
4. Enzyme substrate is added	4. Enzyme-labelled antiglobulin specific for antibody used in step 3 is added
5. Substrate hydrolysis product is proportional to the amount of antigen present	5,6 Same as 4,5 in direct EIA
Competitive EIA	
Enzyme-Labelled Antigen Method	**Enzyme-Labelled Antibody Method**
1. Antigen-specific antibody is	1. Antigen is attached to solid

Table II Continued

attached to solid phase	phase (may require use of specific antibody)
2. Test specimen containing antigen is added	2. Test specimen containing antigen plus enzyme-labelled specific antibody is added
3. Enzyme-labelled antigen is added	3,4. Same as 4,5 in enzyme-labelled antigen method
4. Enzyme substrate is added	
5. Substrate hydrolysis product is inversley proportional to the amount of antigen present	

TABLE III

Quantitation of Immunoglobulin Immobilization on Plasticsa

Immunoglobulin (Ig) bound	Solid-phase support	Immobilizatin technique	Maximum Ig bound (ng/mm^2)
Sheep IgG	polystyrene (latex)	covalent linkage	100
Rabbit IgG	nylon beads	covalent linkage	590
Rabbit IgG	polystyrene (latex)	adsorption	5.7
Rat Ig	poymethyl-metoacrylic beads	adsorption	0.9
Bovine IgG1	polystyrene tubes	adsorption	3.2
Bovine IgM	polystyrene tubes	adsorption	2.9
Human IgG	polystyrene (latex)	adsorption	3.6

aAdapted from Herrmann (1981).

Table IVA

Enzyme Immunoassay for Serodiagnosis:

Bacterial and Mycotic Infections

Microbial Agent	Primary Reference
Bacteria	
Bacillus anthracis	Johnson-Winegar (1984).
Bacteroides fragilis	Rissing et al. (1979).
Bordetella pertussis	Vijanen et al. (1982).
Brucells abortus	Magee (1980).
Chlamydia trachomatis	Rai, et al. (1983);Duc-Goiren (1983).
Clostridium tetani (toxin)	Haberman and Heller (1976).
Corynebacterium diphtheriae (toxin)	Svenson and Larsen (1977).
Escherichia coli (toxin)	Jodal, et al. (1974).
Francisella tularensis	Carlsson, et al. (1979).
Legionella pneumophila	Farshy, et al. (1978).
Leptospira icterohemorragica	Adler, et al. (1980).
Lyme Disease spirochete	Craft, et al. (1984)
Mycobacterium tuberculosis	Kalish, et al. (1984).
Mycobacterium leprae	Douglas and Worth (1984).
Mycoplasma hominis	Miettinen, et al. (1983).
Mycoplasma pneumoniae	Raisanen, et al. (1980).

(Table IVA continued)

Neisseria gonorrhoeae	Ison, et al. (1981).
Neisseria meningitidis	Sippel, et al. (1980).
Salmonella sp.	Carlsson, et al. (1972).
Shigella dysenteriae	Lindberg, et al. (1984).
Staphylococcus aureus	Mackowiak and Smith (1978).
Streptococcus group A	Russel, et al. (1976).
Treponema pallidum	Veldkamp and Visser (1975).
Ureaplasma urealyticium	Wiley and Quinn (1984).
Vibrio cholerae (toxin)	Majumbar, et al. (1981).
Yersinia enterocolitica	Granfors, et al. (1981).
Yersinia pestis	Cavanaugh, et al. (1979).

Fungi and Actinomycetes

Candida albicans	Hommel, et al. (1976).
Aspergillus fumigatus	Hommel, et al. (1976).
Nocardia brasiliensis	Zlotnick, H., et al. (1984).
Paracoccidioides brasiliensis	Mendes-Giannini, et al. (1984).

Table IVB

Enzyme Immunoassays for Serodiagnosis: Viral and Rickettsial infections

Virus or Rickettsia	Primary Reference
Adenovirus	Voller, et al. (1976).
Coxsackievirus	Voller, et al. (1976).
Cytomegalovirus	Voller and Bidwell, (1976.
Dengue virus	Dittmar, et al. (1979).
Epstein-Barr virus	Hopkins, et al. (1982).
Hepatitis A virus	Mathiesen, et al. (1978).
Hepatitis B virus	Feinstone, et al. (1979).
Herpes simplex virus	Gilman and Docherty (1977).
Influenza A virus	Leinikki and Passila (1977).
Japanese Encephalitis virus	Burke, et al. (1982).
Lassa fever virus	Niklasson, et al. (1984).
Measles virus	Voller and Bidwell (1976).
Mumps virus	Ukkonen, et al. (1980).
Parainfluenzae b	Drow, et al. (1979);Pepple, et

(Table IVB Continued)

al.,(1980), Sippel, et al.(1984).

Rabies virus	Atanasiu, et al. (1977).
Rickettsia typhi	Halle, et al. (1977).
Rochalimia quintana	Herrmann, et al. (1977).
Rift Valley Fever virus	Niklasson, et al. (1984).
Ross River Virus	Oseni, et al. (1983).
Rotavirus	Yolken, et al. (1978).
Rubella virus	Gravell, et al. (1977).
St. Louis Encephalitis virus	Monath, et al. (1984).
Tick-borne Encephalitis virus	Hofmann, et al. (1979).
Varicella-Zoster virus	Forghani, et al. (1978).

Table VA

Enzyme Immunoassays for the Detection of Microbial Antigens in Clinical Specimens: Bacteria and Fungi

Microbial Agent	References
Bacteria	
Bacteroides fragilis	Rissing, et al. (1984).
Chlamydia trachomatis	Herrmann, et al. (1983);Jones et al. (1984), Stokes and Khan (1984).
Clostridium botulinum(toxins)	Dezfulian, et al. (1984).
Clostridium deficile(toxins)	Lyerly, et al. (1983), Laughon, et al. (1984).
Hemophilus influenzae b	Drow, et al.(1979); Pepple, et al.(1980), Sippel, et al.(1984).
Legionella pneumophila	Sathapatayavongs, et al. (1982); Berdel, et al. (1979); Bibb, et al. (1984).
Mycobacterium sp.	Sada, et al. (1983).
Mycoplasma hominis	Miettinen, et al. (1984).
Neisseria gonorrhoeae	Aardoom, et al. (1982); Burns, et al.(1983); Danielsson, et al.(1983); Papasian, et al, (1984) a,b; Martin, et al. (1984), Schacter, et al. (1984), Stamm, et al. (1984).

(Table VA, continued)

	Nachamkin, et al.(1984); Demetriou , et al. (1984); Manis, et al. (1984).
Neisseria meningitidis	Sippel and Voller, (1980); Sippel, et al. (1984); Sugasawara, et al, (1984).
Streptococcus group A	Knigge, et al. (1984).
Streptococcus pneumoniae	Sippel, et al. (1984); Yolken, et al. (1984).
Yersinia pestis	Williams, et al. (1984).
Fungi	
Candida albicans	Segal, et al. (1979).

Table VB

Enzyme Immunoassays for the Detection of Microbial Antigens in Clinical Specimens: Viruses

Virus	References
Adenovirus	Sarkkinen, et al. (1980);Johansson, et al. (1980); Sarkkinen, et al. (1981c); Harmon and Pawlick, (1982).
Coronavirus	Macnaughton, et al. (1983).
Coxsackievirus	Yolken and Torsch, (1980, 1981).
Cytomegalovirus	Pronovost, et al. (1982).
Hepatitis A virus	Mathiesen, et al. (1978).
Hepatitis B virus	Wolters, et al. (1976).
Herpes Simplex virus	Miranda, et al. (1977); Grillner and Landqvist, (1983), Land, et al.(1984), Lawrence, et al.(1984), Morgan and

(Table VB continued)

	Smith, (1984); Nerurkar, et al.(1984); Warford, et al. (1984).
Influenza A virus	Berg, et al, (1980); Harmon, et al (1983), Sarkkinen, et al. (1981b).
Parainfluenza virus	Sarkkinen, et al. (1981 a,c).
Respiratory Syncytial virus	Chao, et al. (1979); Sarkkinen, et al. (1981c); Hornsleth, et al. (1981); McIntosh, et al. (1982); Meurman, et al. (1984).
Rotavirus	Yolken, et al. (1977); (1980); Sarkkinen, et al. (1980)
Varicella-Zoster virus	Ziegler (1984).